be combined in equal volume-density and stored in the refrigerator (5° to 10° C.) in tightly stoppered bottles.

(f) The diluted antigen to be used in the routine testing should be prepared from the stock antigen by dilution of the latter with physiological (0.85 percent) saline solution containing 0.25 percent of phenol to a turbidity corresponding to 0.75–1.00 on the McFarland nephelometer scale. The hydrogen-ion concentration of the diluted antigen should be corrected to pH 8.2 to 8.5 by the addition of dilute sodium hydroxide. New diluted antigen should be prepared each day and kept cold. The diluted antigen may be employed in 2 cc. quantities in 4 by ½–inch test tubes, or 1 cc. quantities in smaller tubes, in which the final serum-antigen mixtures are made and incubated. The distribution of the antigen in the tubes may be accomplished by the use of long burettes, or special filling devices made for the purpose.

(g) The maximum serum dilution employed must not exceed 1:50 for chickens, nor 1:25 for turkeys. The available data indicate that 1:25 dilution is the most efficient. In all official reports on the blood test, the serum dilutions shall be indicated. The sera should be introduced into the agglutination tubes in the desired amounts with well-cleaned serological pipettes or special serum-delivery devices which do not permit the mixing of different sera. The antigen and serum should be well mixed before incubation. The serum and antigen mixture must be incubated for at least 20 hours at 37° C.

(h) The results shall be recorded as:

N, or − (negative) when the serum-antigen mixture remains uniformly turbid.

P, or + (positive) when there is a distinct clumping of the antigen, and the liquid between the agglutinated particles is clear.

S, or ? (suspicious) when the agglutination is only partial or incomplete.

M, or missing, when samples listed on the original record sheet are missing.

H, or hemolyzed, when blood samples are hemolyzed and cannot be tested.

B, or broken, when sample tubes are broken and no serum can be obtained.

(Some allowance must always be made for the difference in sensitiveness of different antigens and different set-ups, and therefore, a certain amount of independent, intelligent judgment must be exercised at all times. Also, the histories of the flocks require consideration. In flocks where individuals show a suspicious agglutination, it is desirable to examine representative birds bacteriologically to determine the presence or absence of S. pullorum.)

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[36 FR 23121, Dec. 3, 1971. Redesignated at 44 FR 61586, Oct. 26, 1979, as amended at 59 FR 12799, Mar. 18, 1994]

§147.2 The rapid serum test.[2]

(a) The procedure for the collection and delivery of blood samples in the rapid serum test is the same as that described in §147.1(a).

(b) The selection and maintenance of suitable strains of S. pullorum and the composition of a satisfactory medium are described in §147.1 (b) and (c).

(c) Large 1–inch test tubes, Kolle flasks, or Blake bottles are streaked liberally from 48–hour slant-agar cultures prepared from stock cultures of the selected strains.

(d) The antigen-growing tubes or bottles should be incubated 48 hours at 37° C., and the surface growth washed off with a very slight amount of 12 percent solution of sodium chloride containing 0.25 to 0.5 percent phenol, filtered through lightly packed sterile absorbent cotton placed in the apex of a sterile funnel.

(e) The washings should be adjusted (using 12 percent sodium chloride containing 0.25 to 0.5 percent phenol) so that the turbidity is 50 times greater than tube 0.75 of McFarland's nephelometer, or to a reading of 7 mm. by the Gates nephelometer.

(f) The individual strain antigens should be tested with negative sera for

[2] The procedure described is a modification of the method reported by Runnels, Coon, Farley, and Thorpe, Amer. Vet. Med. Assoc. Jour. 70 (N.S. 23): 660–662 (1927).

their insensitivity and with positive sera for high agglutinability in comparison with known satisfactory antigen. The antigens of the separate strains should be combined in equal volume-density and stored in the refrigerator (5° to 10° C.) in tightly stoppered bottles.

(g) The tests should be conducted on a suitable, smooth plate. The serum-antigen dilution should be made so that the dilution will not exceed 1:50 when compared to the standard tube agglutination test. When testing turkey blood samples, it is desirable to use a serum-antigen dilution equivalent to the 1:25 in the tube method. The serum should be added to the antigen and mixed thoroughly by use of the tip of the serum pipette. Most strong positive reactions will be plainly evident within 15 to 20 seconds. The final reading should be made at the end of 2 or 3 minutes. Heating the plate at approximately 37° C. will hasten agglutination. Before reading, the plate should be rotated several times.

(h) The results shall be recorded as described in § 147.1(h).

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[36 FR 23121, Dec. 3, 1971. Redesignated at 44 FR 61586, Oct. 26, 1979, as amended at 59 FR 12799, Mar. 18, 1994]

§ 147.3 The stained-antigen, rapid, whole-blood test.[3]

(a) The description of the preparation of antigen is not herein included because the antigen is a proprietary product produced only under license from the Secretary of Agriculture.

(b) A loop for measuring the correct quantity of blood can usually be obtained from the manufacturer of the antigen. A satisfactory loop may be made from a piece of No. 20 gage nichrome wire, 2½ inches long, at the end of which is fashioned a loop three-sixteenths of an inch in diameter. Such a loop, when filled with blood so that the blood appears to bulge, delivers 0.02 cc. A medicine dropper whose tip is adjusted to deliver 0.05 cc. is used to measure the antigen. A glass plate about 15 inches square, providing space for 48 tests, has proved satisfactory for this work. The use of such a plate enables the tester to have a number of successive test mixtures under observation without holding up the work to wait for results before proceeding to the next bird.

(c) A drop of antigen should be placed on the testing plate. A loopful of blood should be taken up from the wing vein. When submerged in the blood and then carefully withdrawn, the loop becomes properly filled. On looking down edgewise at the filled loop, one observes that the blood appears to bulge. The loopful of blood then should be stirred into the drop of antigen, and the mixture spread to a diameter of about 1 inch. The loop then should be rinsed in clean water and dried by touching it to a piece of clean blotting paper, if necessary. The test plate should be rocked from side to side a few times to mix the antigen and blood thoroughly, and to facilitate agglutination. The antigen should be used according to the directions of the producer.

(d) Various degrees of reaction are observed in this as in other agglutination tests. The greater the agglutinating ability of the blood, the more rapid the clumping and the larger the clumps. A positive reaction consists of a definite clumping of the antigen surrounded by clear spaces. Such reaction is easily distinguished against a white background. A somewhat weaker reaction consists of small but still clearly visible clumps of antigen surrounded by spaces only partially clear. Between this point and a negative or homogeneous smear, there sometimes occurs a very fine granulation barely visible to the naked eye; this should be disregarded in making a diagnosis. The very fine marginal clumping which may occur just before drying up is also regarded as negative. In a nonreactor, the smear remains homogeneous. (Allowance should be made for differences in the sensitiveness of different antigens and different set-ups, and therefore, a certain amount of independent, intelligent judgment must be exercised at all times. Also, the histories of the flocks require consideration. In flocks

[3] The procedure described is a modification of the method reported by Schaffer, MacDonald, Hall, and Bunyea, Jour. Amer. Vet. Med. Assoc. 79 (N. S. 32): 236–240 (1931).